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APPLICATION NUMBER: 60/513,355
FILING DATE: *October 22, 2003*
RELATED PCT APPLICATION NUMBER: PCT/US04/35363

Certified by



Jon W Dudas

Acting Under Secretary of Commerce
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and Acting Director of the U.S.
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 971287301 US

INVENTOR(S)					
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)			
Albert J. Melissa Marie	Banes Maloney	Hillsborough, NC Carrboro, NC			
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Automated System for Imaging Artificial Tissue Constructs in a Controlled Environment					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number <div style="border: 1px solid black; display: inline-block; padding: 2px 10px;">28289</div> <div style="margin-left: 20px;">→</div> <div style="border: 1px solid black; display: inline-block; padding: 2px 10px; text-align: center;"> Place Customer Number Bar Code Label here </div>		<div style="writing-mode: vertical-rl; transform: rotate(180deg);">15535 U.S. PTO</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">60/51338</div> <div style="writing-mode: vertical-rl; transform: rotate(180deg);">10/22/03</div>			
OR <div style="display: flex; align-items: center;"> <div style="margin-right: 10px;"> <input type="checkbox"/> Firm or Individual Name </div> <div>Webb Ziesenheim Logsdon Orkin & Hanson, P.C.</div> </div>					
Address		700 Koppers Building			
Address		436 Seventh Avenue			
City	Pittsburgh	State	Pennsylvania	Zip	15219
Country	U.S.A.	Telephone	412-471-8815	Fax	412-471-4094
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages <div style="border: 1px solid black; display: inline-block; padding: 2px 10px;">2</div>		<input type="checkbox"/> CD(s), Number <div style="border: 1px solid black; display: inline-block; width: 60px; height: 20px;"></div>			
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets <div style="border: 1px solid black; display: inline-block; padding: 2px 10px;">33</div>		<input type="checkbox"/> Other (specify) <div style="border: 1px solid black; display: inline-block; width: 150px; height: 30px;"></div>			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE	
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees				AMOUNT (\$)	
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: <div style="border: 1px solid black; display: inline-block; padding: 2px 10px;">23-0650</div>				<div style="border: 1px solid black; display: inline-block; padding: 2px 10px;">\$80.00</div>	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

Respectfully submitted,

Date

October 22, 2003

SIGNATURE

TYPED or PRINTED NAME

Nathan J. Prepelka

TELEPHONE

412-471-8815

REGISTRATION NO.
(if appropriate)

43,016

Docket Number:

717-031951

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the PTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

ATTORNEY'S DOCKET NUMBER

ALBERT J. BANES and
MELISSA MARIE MALONEY

717-031951

ENTITLED

**AUTOMATED SYSTEM FOR IMAGING ARTIFICIAL TISSUE CONSTRUCTS
IN A CONTROLLED ENVIRONMENT**

Mail Stop PROVISIONAL PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

EXPRESS MAIL CERTIFICATE

"Express Mail" Label Number EL 971287301 US

Date of Deposit October 22, 2003

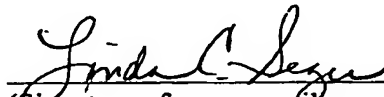
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- PROVISIONAL APPLICATION FOR PATENT COVER SHEET (1 p. in trip.)
- SPECIFICATION (2 pp.)
- DRAWINGS (33) SHEETS
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Linda C. Seger

(Typed name of person mailing paper or fee)



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AUTOMATED SYSTEM FOR IMAGING ARTIFICIAL TISSUE CONSTRUCTS IN A CONTROLLED ENVIRONMENT

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The system of the present invention is directed to a computer-implemented method and system for imaging tissue constructs in a controlled environment in the field of tissue engineering and cell biology, but could be used in any applications where programmable, automated imaging functions would be useful.

2. Background of the Invention

[0002] In the field of tissue engineering, changes in 3D cell-matrix constructs over time are frequently studied. Typically, the cells within these constructs will begin to form attachments within a day after plating and will reorganize and contract the matrix within a few days. Measurements of matrix contraction under the influence of various physical and biochemical factors indicate the impact of each factor on cellular function.

[0003] Currently, measurements of matrix contraction are performed manually by periodically moving the culture plates from their controlled environment (inside an incubator) to access an external imaging device (camera or scanner). Depending on the effect being measured, this process may need to be repeated every few hours, day and night, for several days. In addition to being labor intensive, this process is also less than ideal for the cell cultures themselves. Preferably, the contraction of the constructs would be monitored without repeatedly exposing the constructs to dramatic environmental changes.

DESCRIPTION OF THE INVENTION

[0004] The system of the present invention incorporates an imaging device and a software program, which together allow the user to automatically monitor matrix contraction without removing the cell cultures from the incubator (see Figure 1). The scanner is set up inside the incubator with the cell culture plates placed on the scanner glass and the controller (computer with custom software, see Figure 2) connected and running nearby. The user inputs the desired scanning parameters (filename and type, resolution, etc), selects the scan area, and creates a scanning regimen. Once the program is launched, it will automatically initiate scans at the times indicated by this regimen, and the culture plates need only be removed from the incubator for steps such as media changes (in lengthy studies). The images are stored to a user-designated folder for future analysis or may, with additional computer processing, be automatically analyzed. Multiple imaging devices can be controlled from a

single computer/application to increase the number of culture plates that can be monitored. With the appropriate imaging devices and lighting variations (visible light, IR, UV), this application could also be extended to monitor changes at the cellular level, including performing analyses based on color distinctions. Block diagrams of the scanning function software (Figs. 3-9) and frequency set up software (Figs 10-17) are attached.

[0005] The computer-implemented method and automated system is embodied in the form of an executable software program, preferably with a Graphical User Interface (GUI). The user interfaces with the GUI and interacts with the method and system of the present invention. The software program of the present invention may also interact with or execute using other enabling and/or proprietary software, such as LabVIEW™ by National Instruments. Various screenshots of the present computer-implemented method, executing in conjunction with LabVIEW™, are illustrated in Figs. 18-27.

EXAMPLE

[0006] Various illustrations, photographs, charts and text created in connection with the following example are illustrated in Figs. 28-34. Human tendon internal fibroblasts (HTIF, 2×10^5 cells/100 μ l/specimen) were plated in linear, tethered, collagen gels in TISSUE TRAIN™ culture plates. After two hours, when the gels had solidified, the culture plates were removed from the FX-4400TT TISSUE TRAIN™ Culture System and placed on the glass of a Plustek OpticPro U24 flatbed scanner. The scanner was configured to collect images every hour for the first four hours of each day and every two hours for the remainder of the day, for four days. Culture plates were only removed from the incubator once a day to change the medium. Images were imported into SigmaScan Software to quantify the area of each gel at each time point. Gels experienced the greatest rate of contraction (58.6%) in the first 24 hours, and continued contracting to a total of 73.6% by the end of Day 4. The measured contraction of the gels followed a trend consistent with contraction results obtained by traditional methods. Besides simplifying the monitoring process, however, experimental results should be more accurate with automated imaging. Any confounding results deriving from changes in temperature/pH are minimized when the cultures can remain in the incubator during imaging. Additionally, the increased number of data points collected improves the resolution of the contraction curve and strengthens the findings. This method can easily be applied to perform contraction analysis with other 3D gel systems, as well.

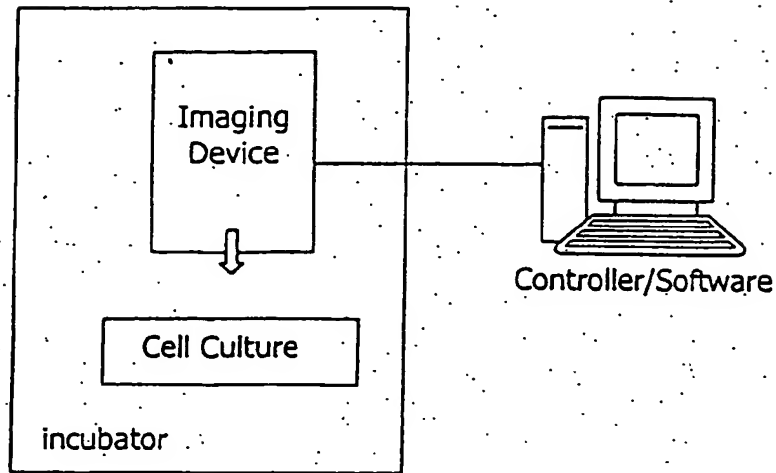


Figure 1: Components of Imaging System

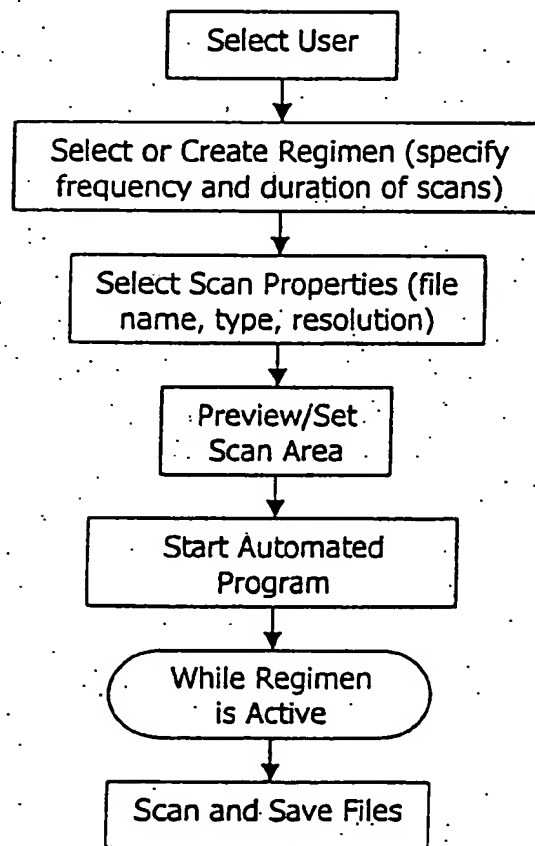


Figure 2: Flowchart of one simple embodiment of the software function

!GUI-Flexcell Scan.vi

Block Diagram

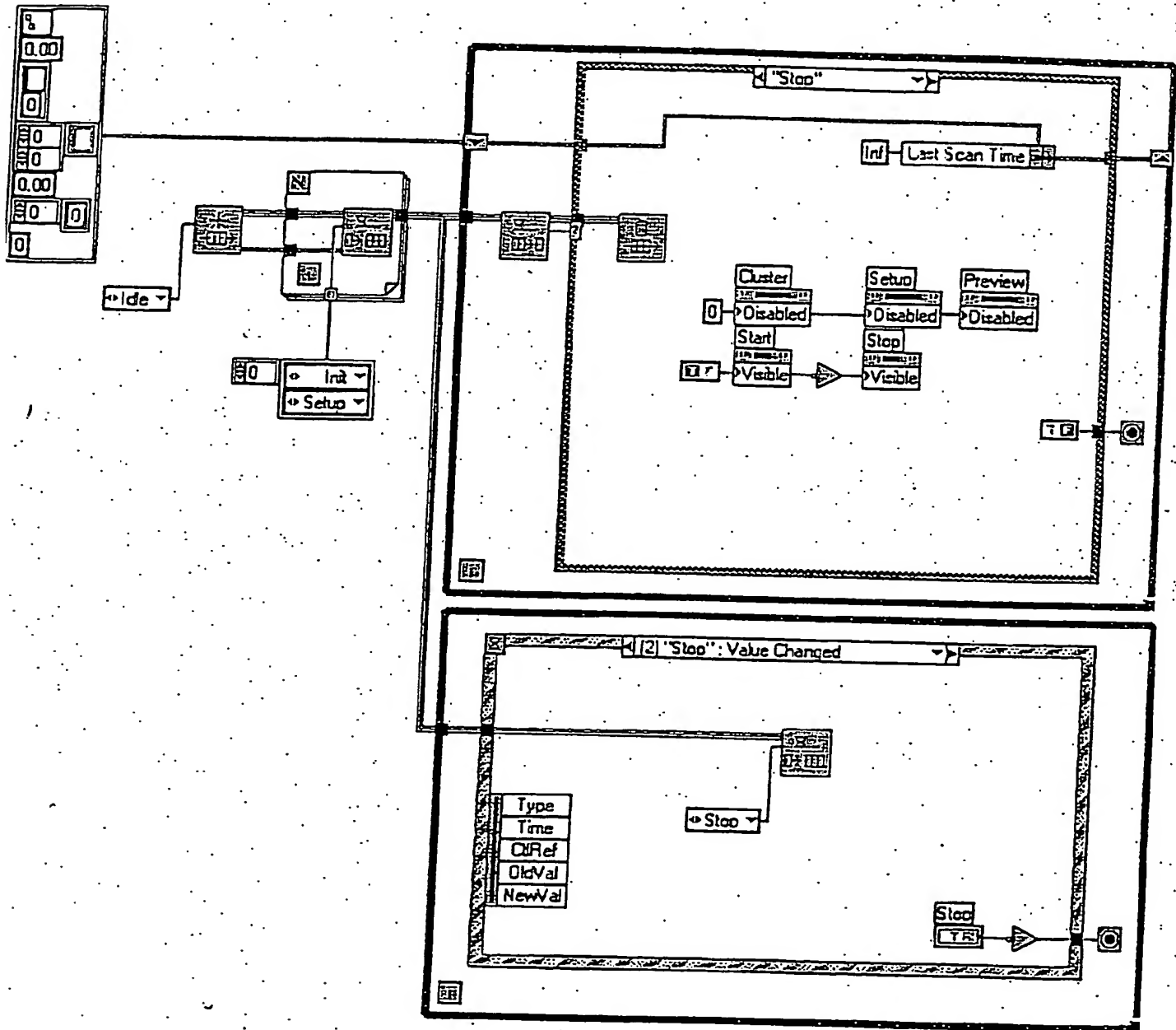


Fig 3

"Automated System for Imaging Artificial Tissue..."

Albert J. BANES et al.

Attorney Docket No. 717-031951

!GUI-Flexcell Scan. vi

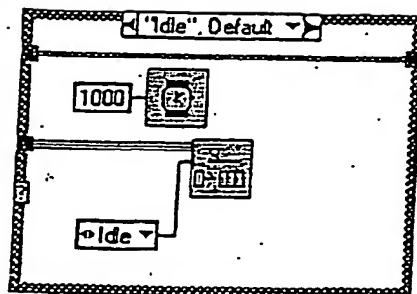
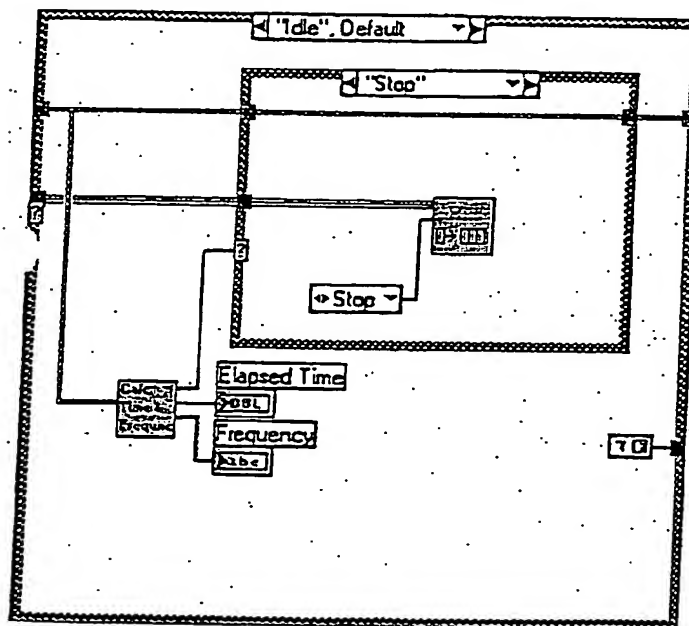
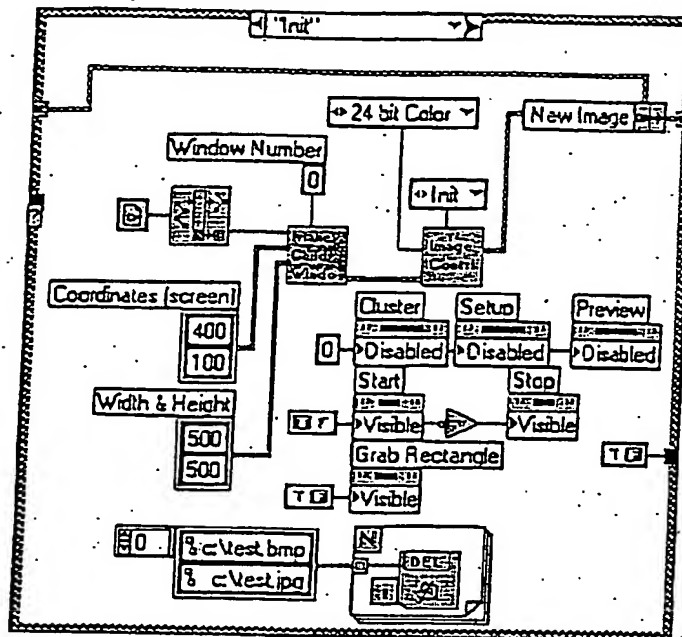


Fig 4

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!GUI-Flexcell Scan.vi

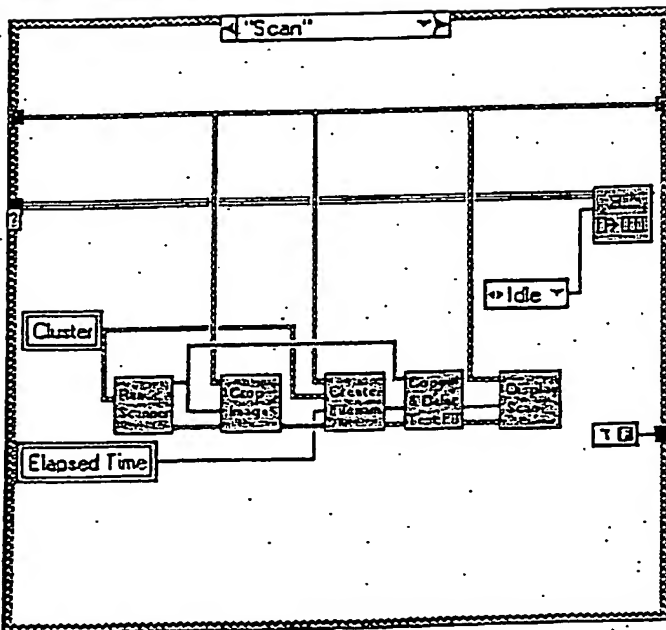
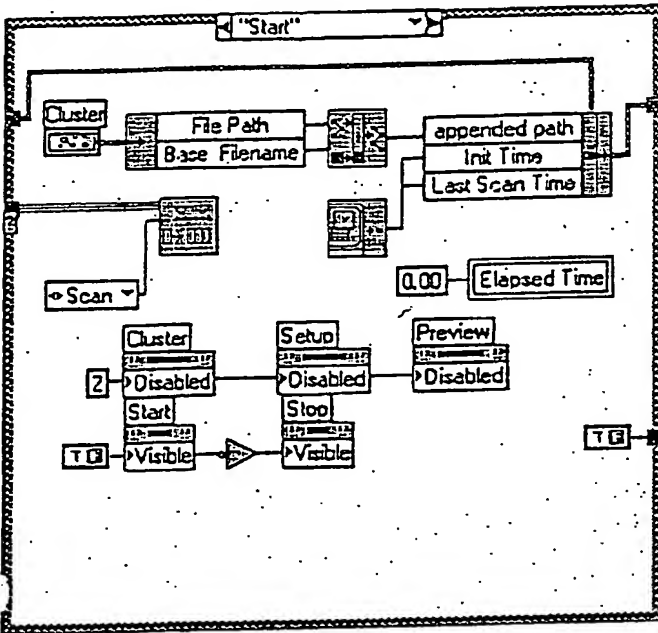
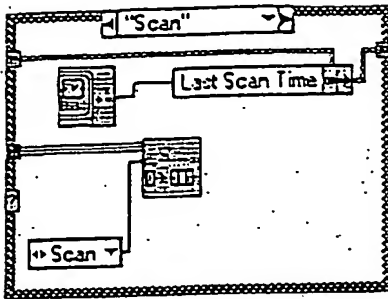


Fig 5

!GUI-Flexcell Scan.vi

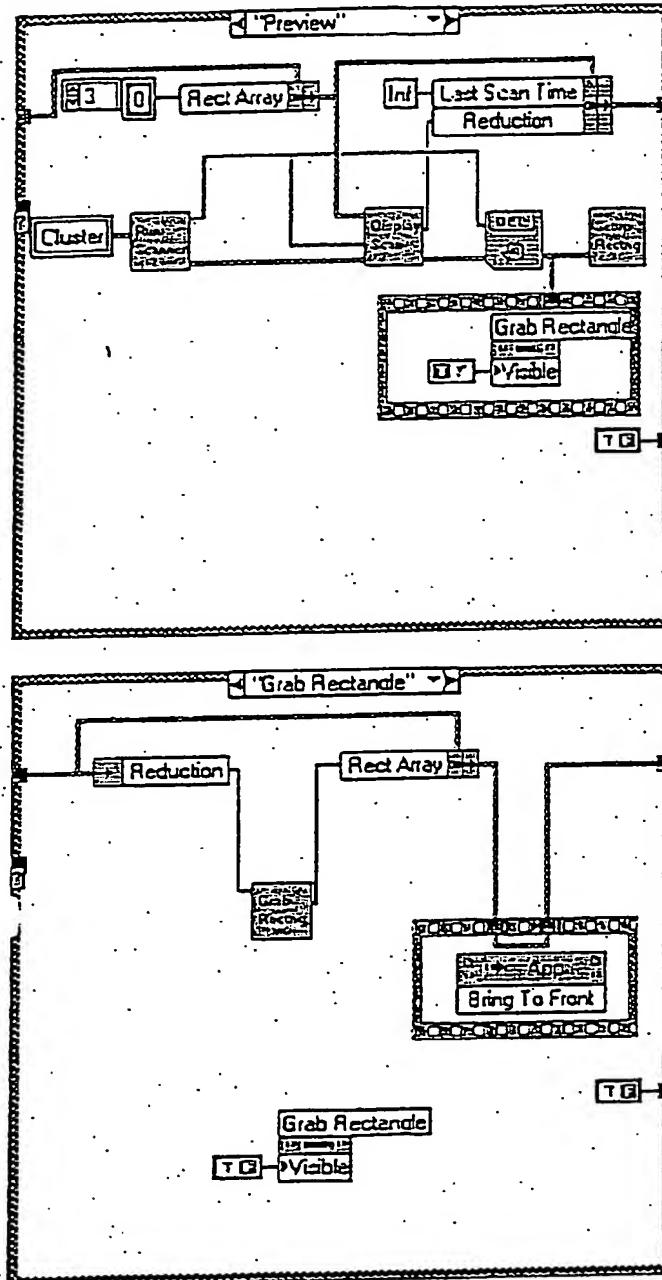


Fig 6

!GUI-Flexcell Scan.vi

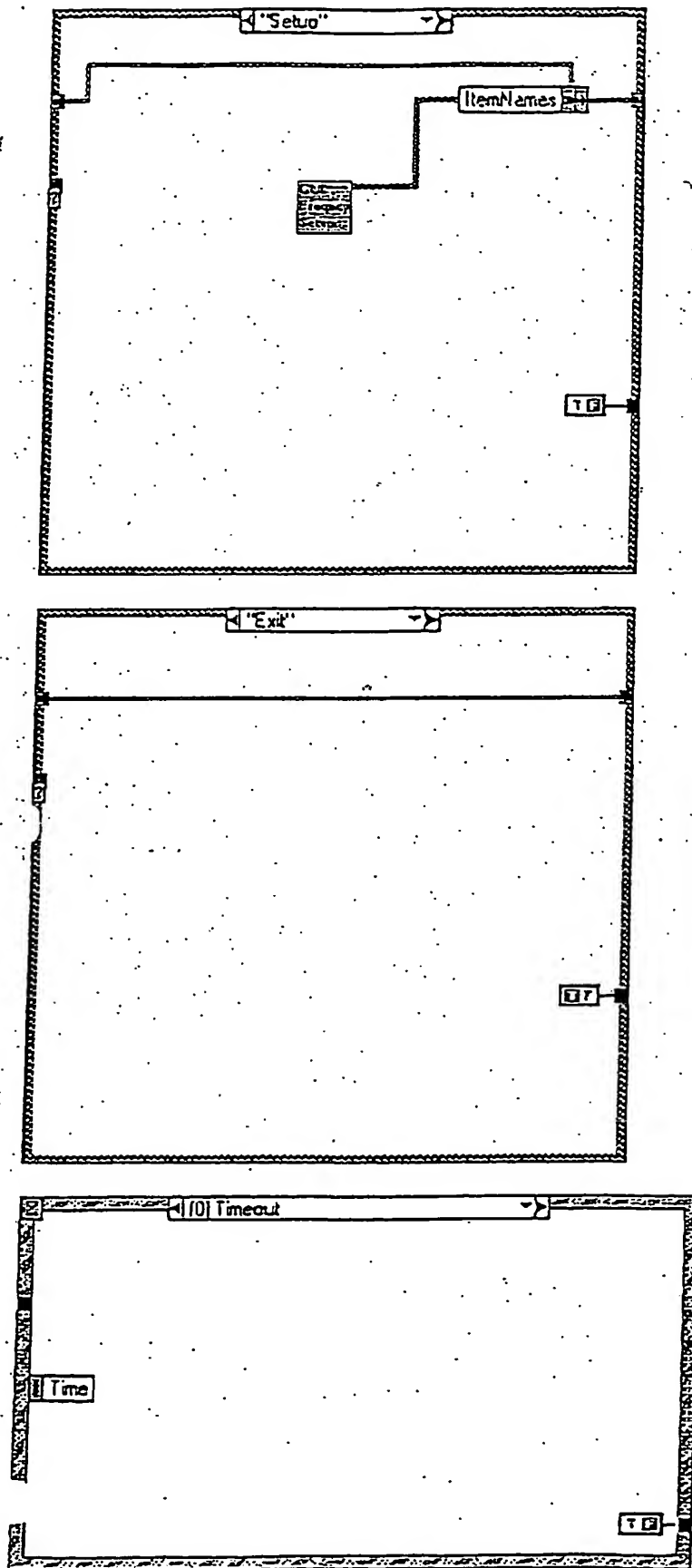


Fig 7

"Automated System for Imaging Artificial Tissue..."

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!GUI-Flexcell Scan.vi

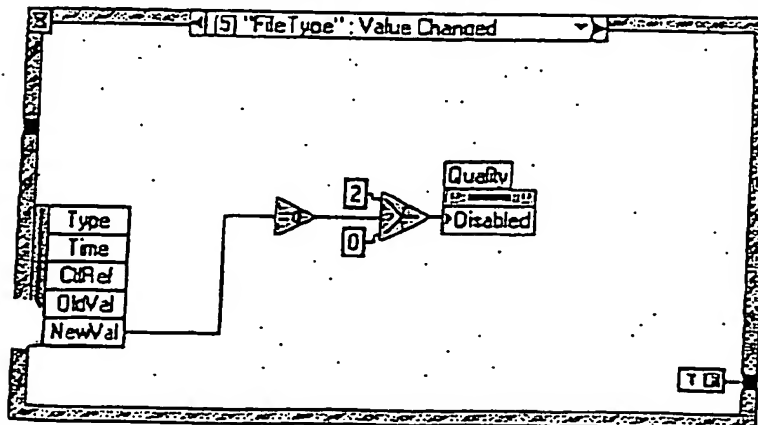
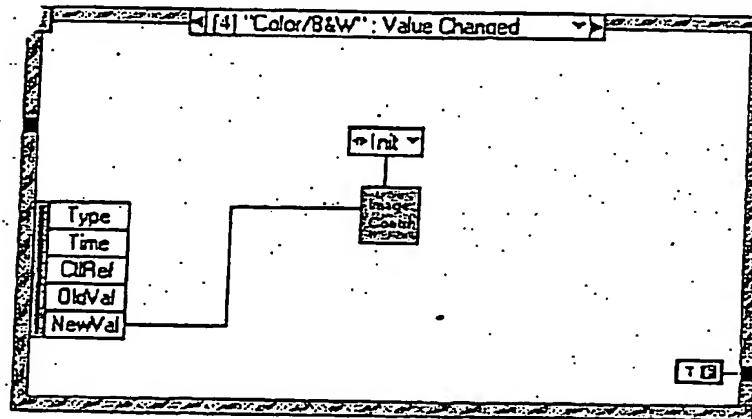
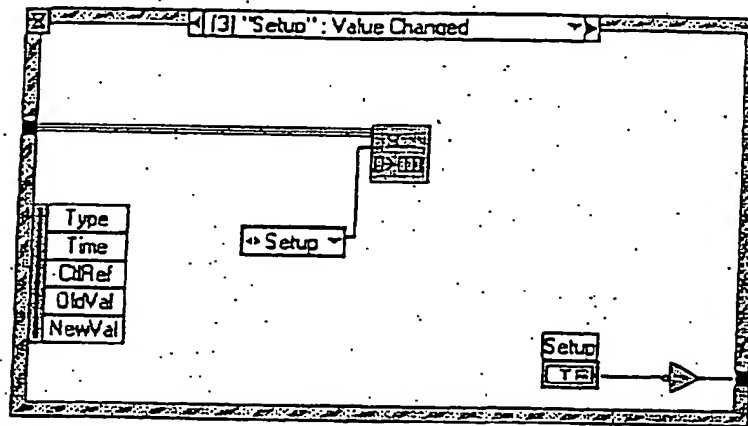
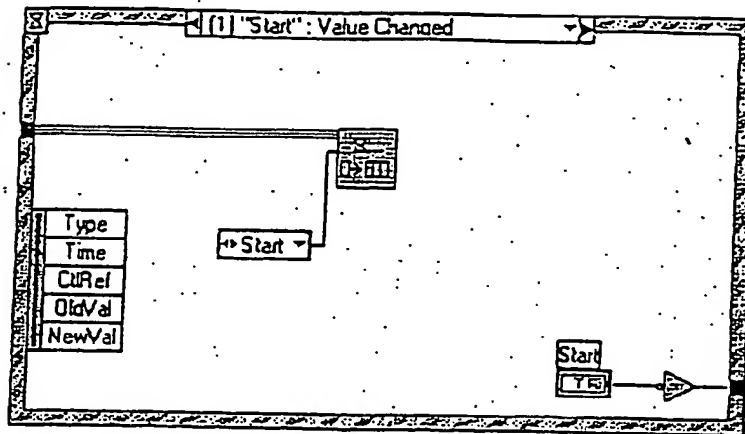


Fig 8

!GUI-Flexcell Scan.vi

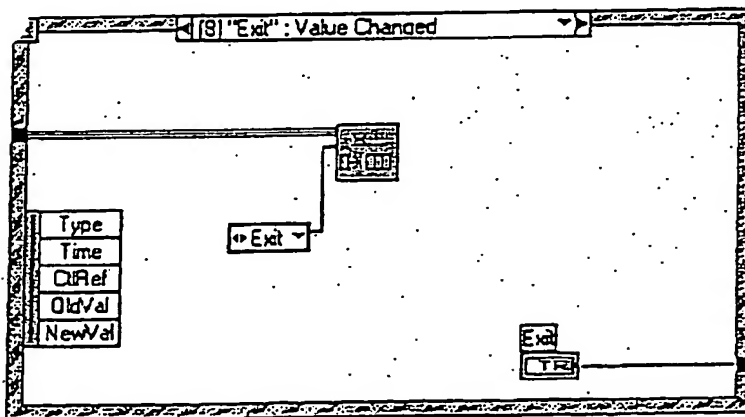
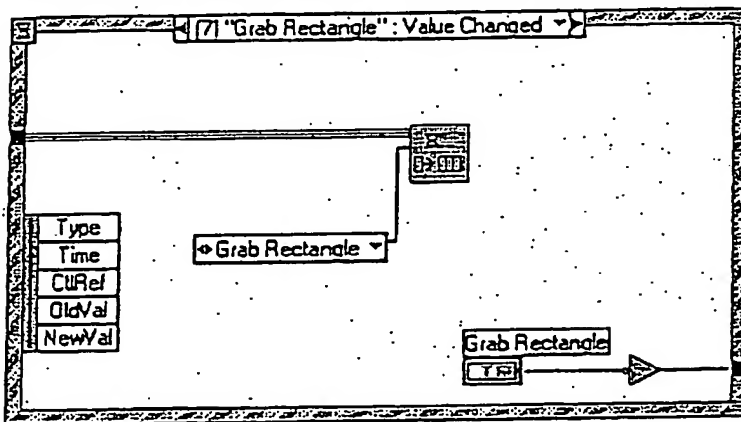
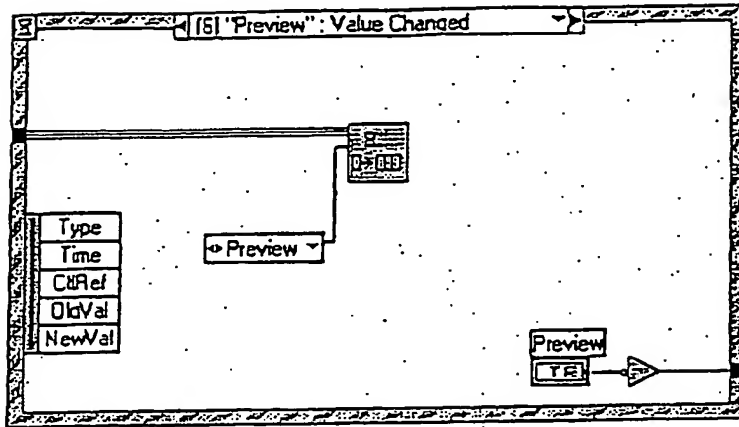


Fig 9

Block Diagram

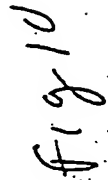


Fig. 11

u01-r1excell frequency Setup. vi

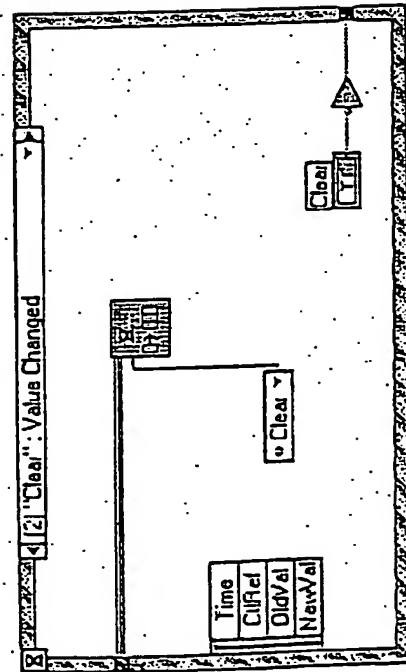
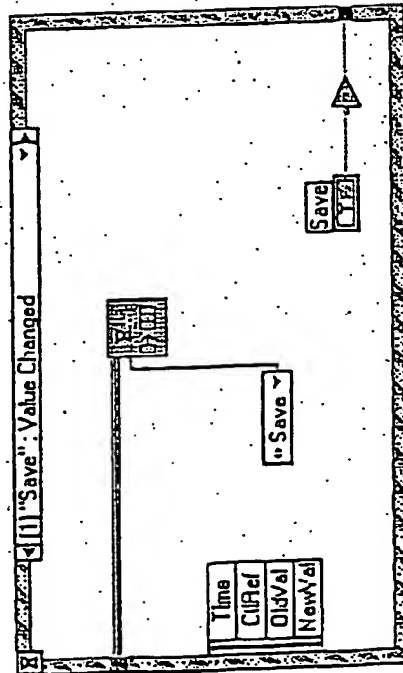
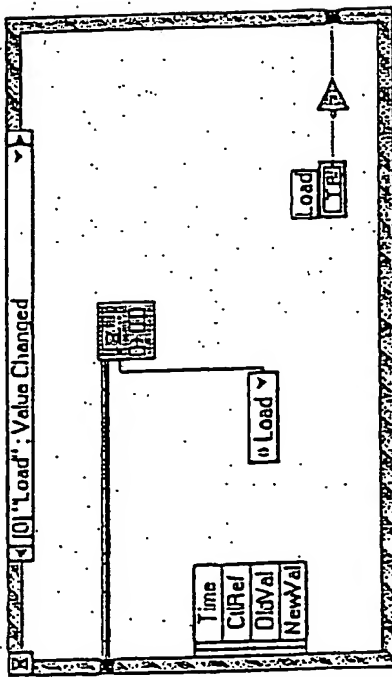


Fig 12

FIG. 12: Frequency Setup.vi

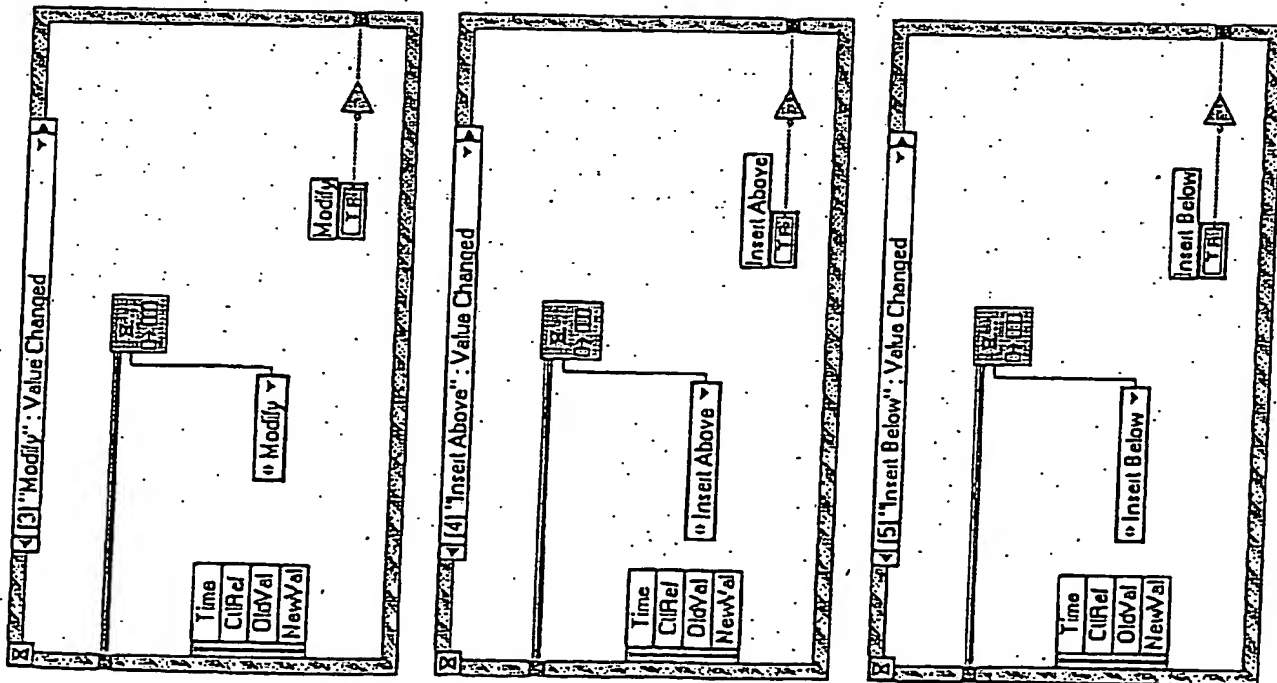
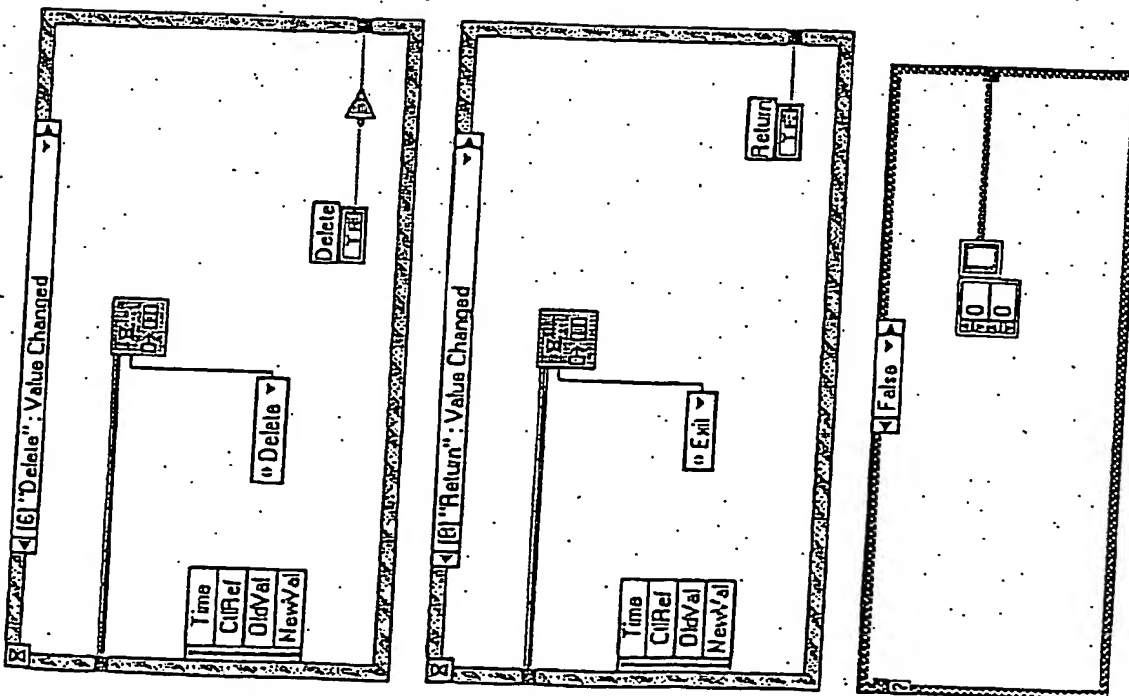


fig 13



"Automated System for Imaging Artificial Tissue..."

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Multi-cell Frequency Setup.vi

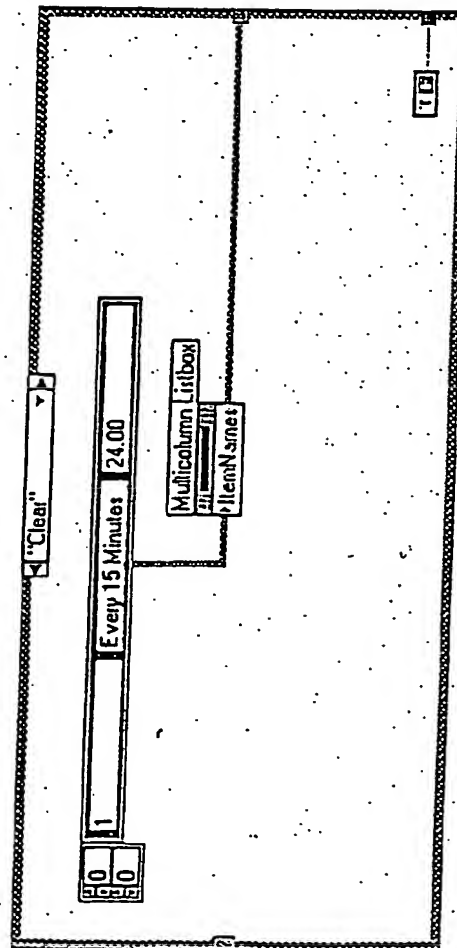
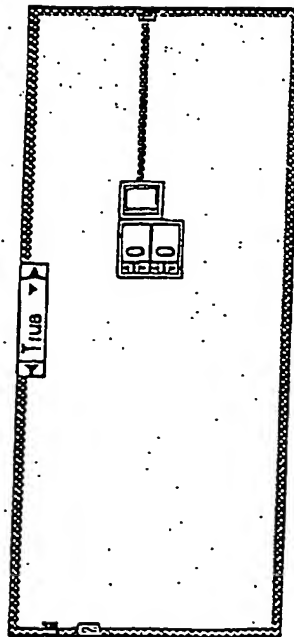
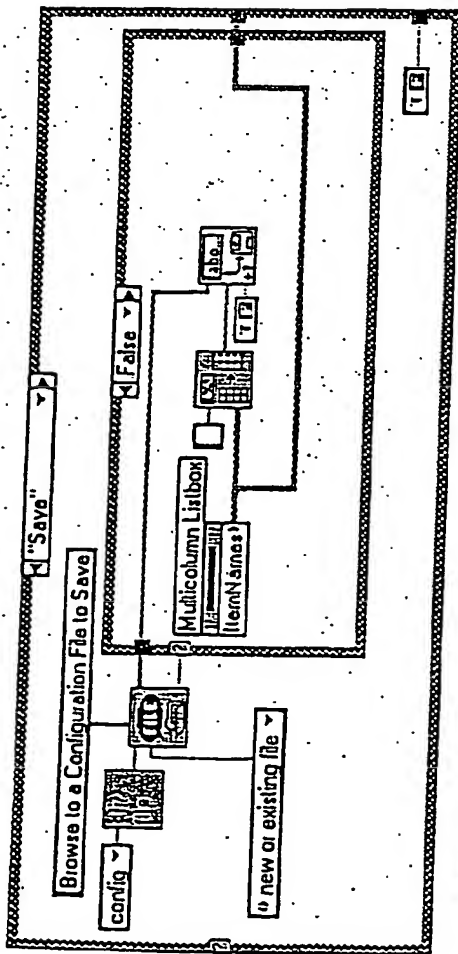
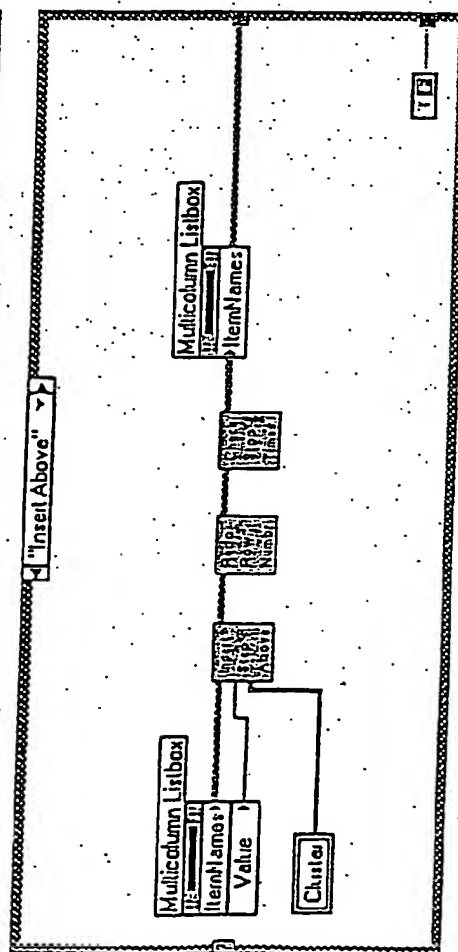
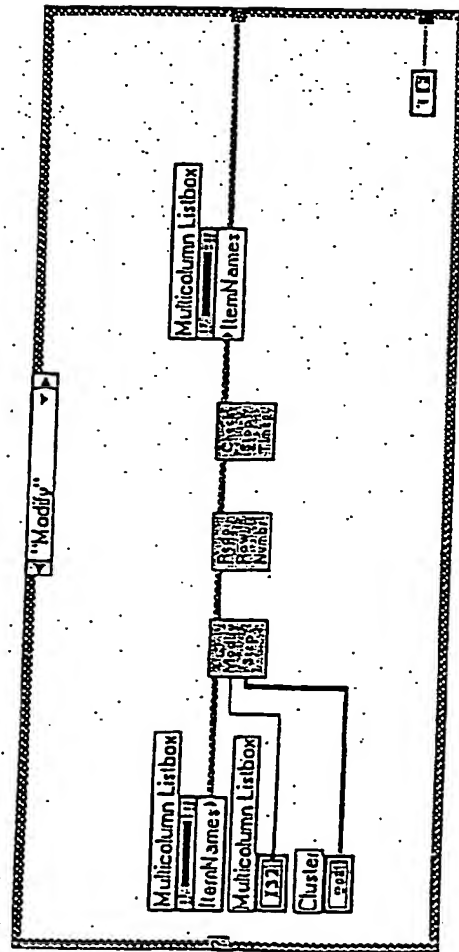
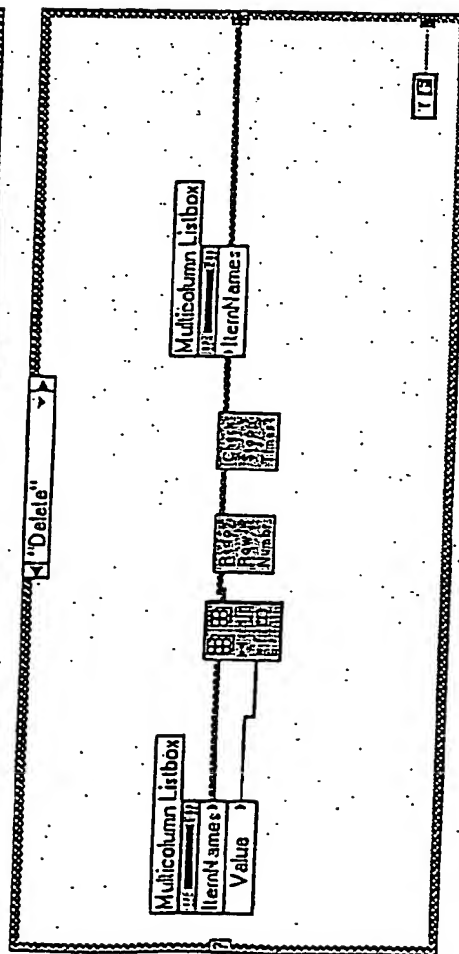
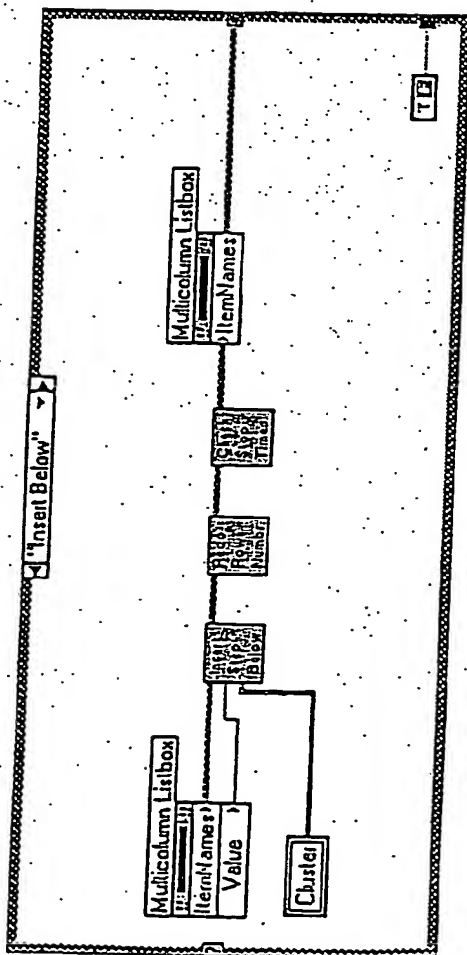


Fig 14

Fig 15

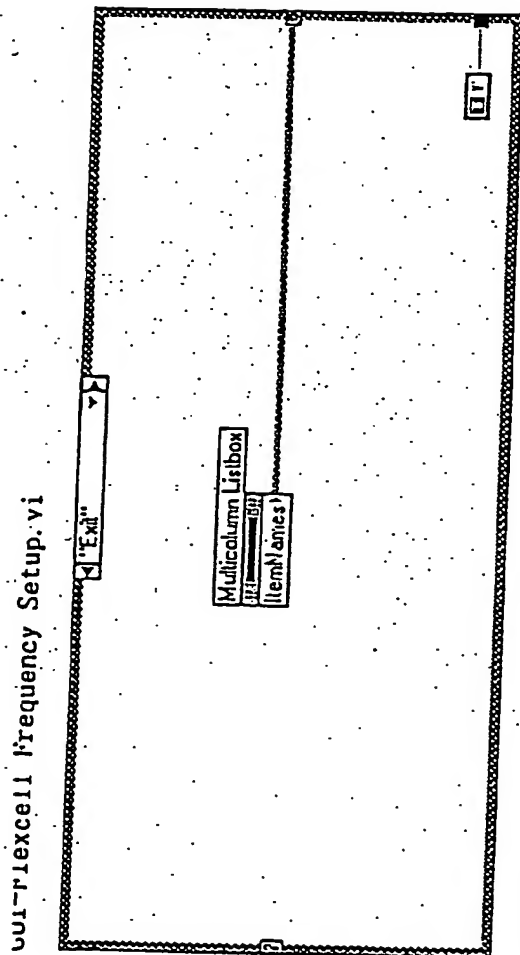
Multi-Column Frequency Setup. vi





619
16

Fig 17



"Automated System for Imaging Artificial Tissue..."

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Attorney Docket No. 717-031951

File Edit Operate Tools Window Help

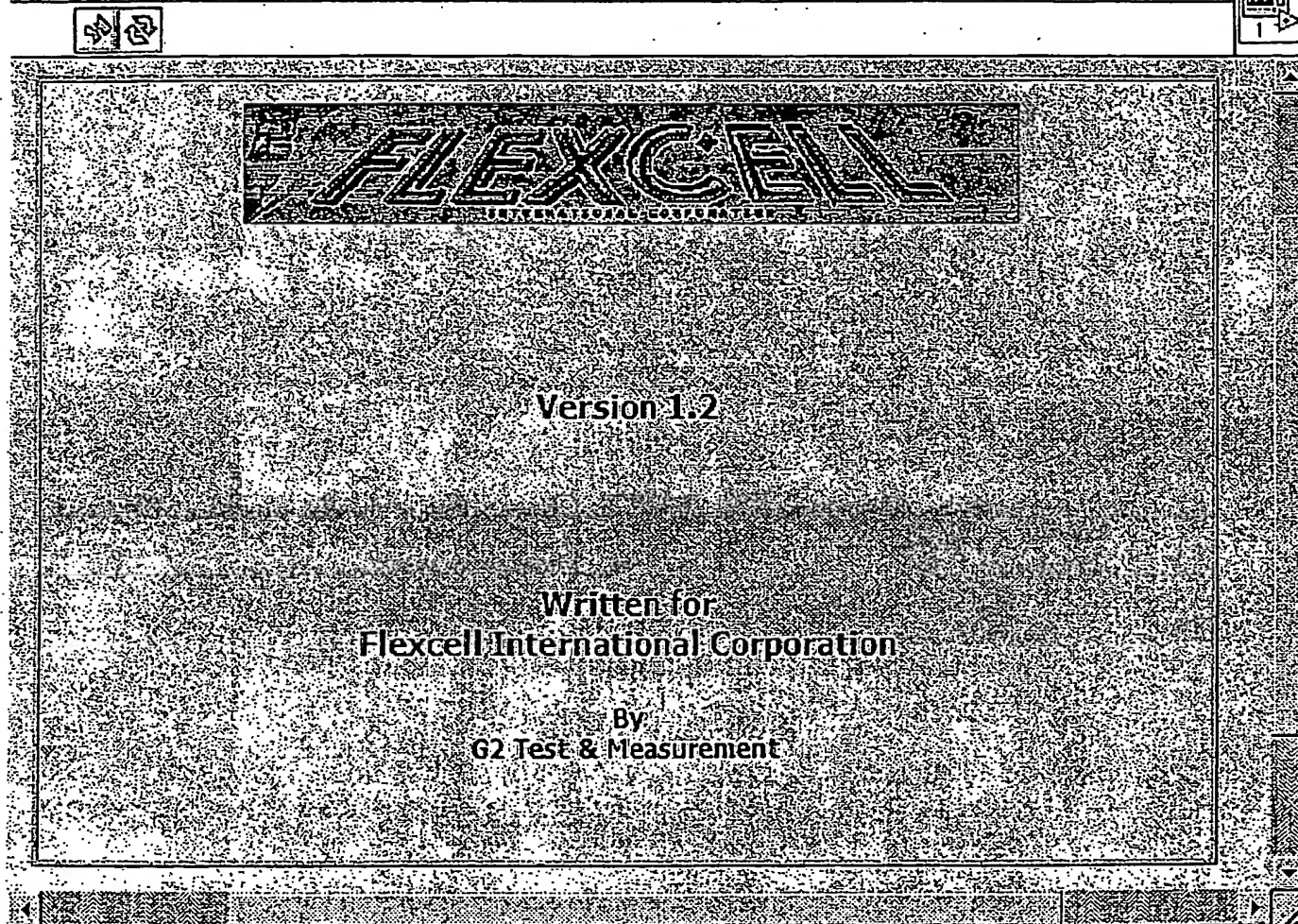


Fig. 18

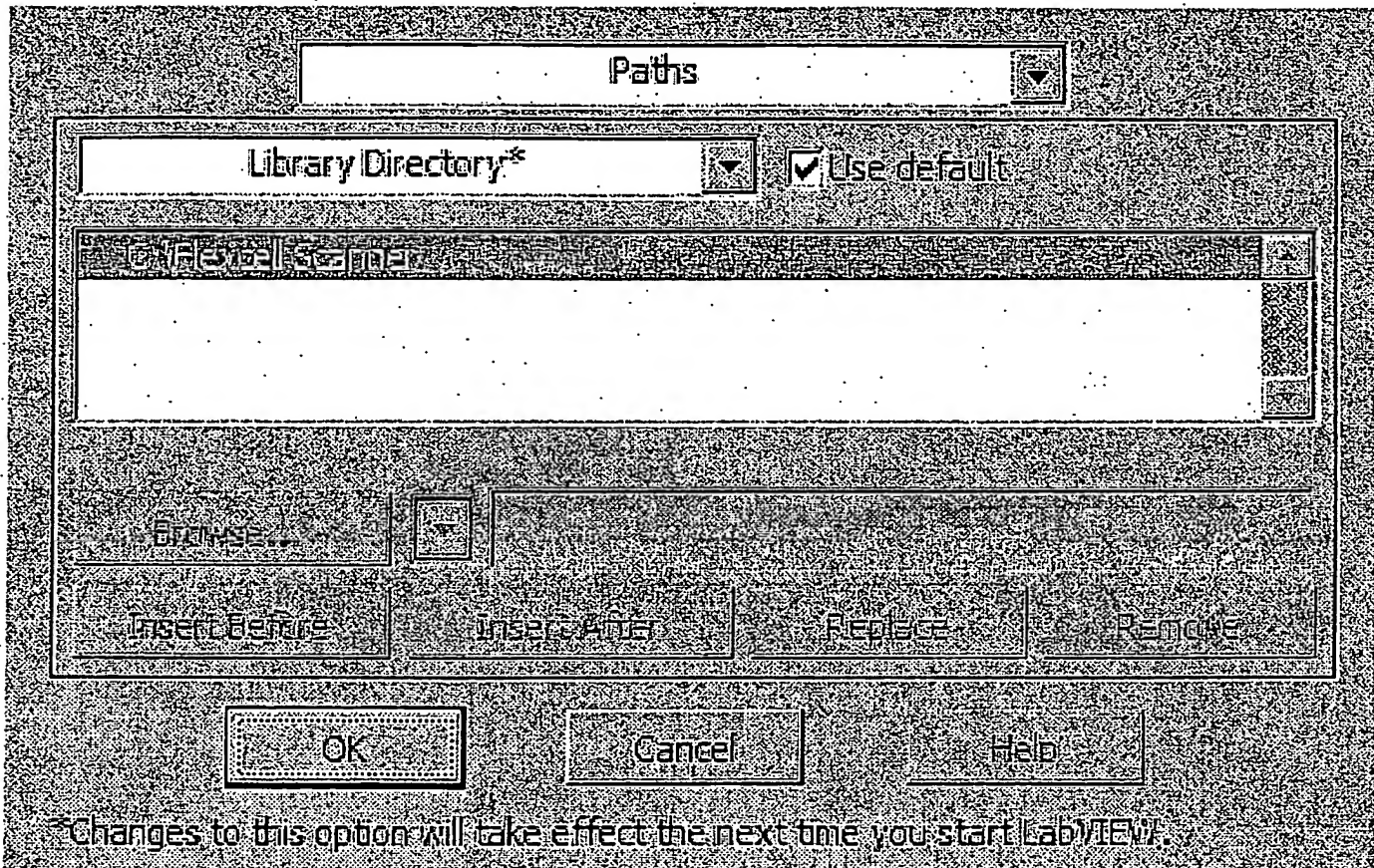


Fig. 19

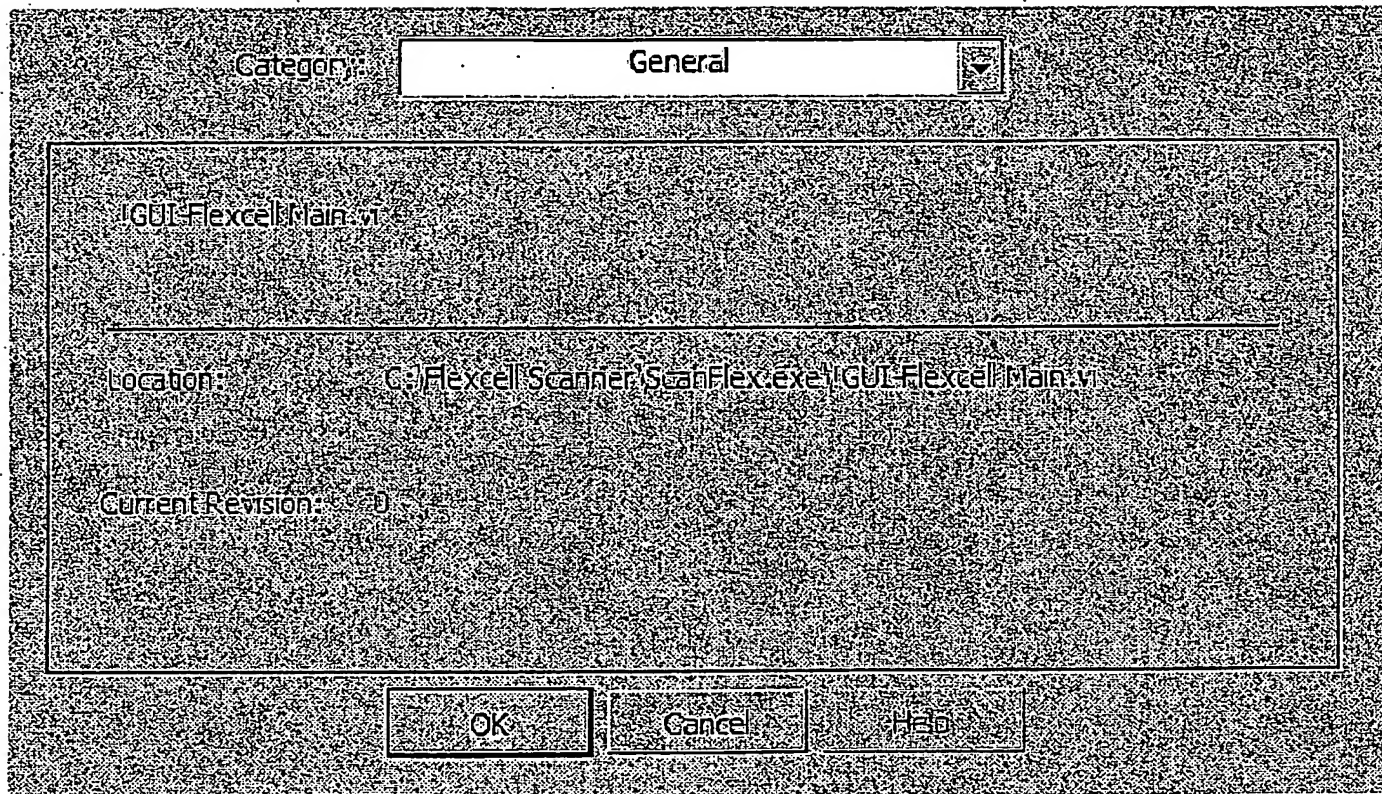


Fig. 20

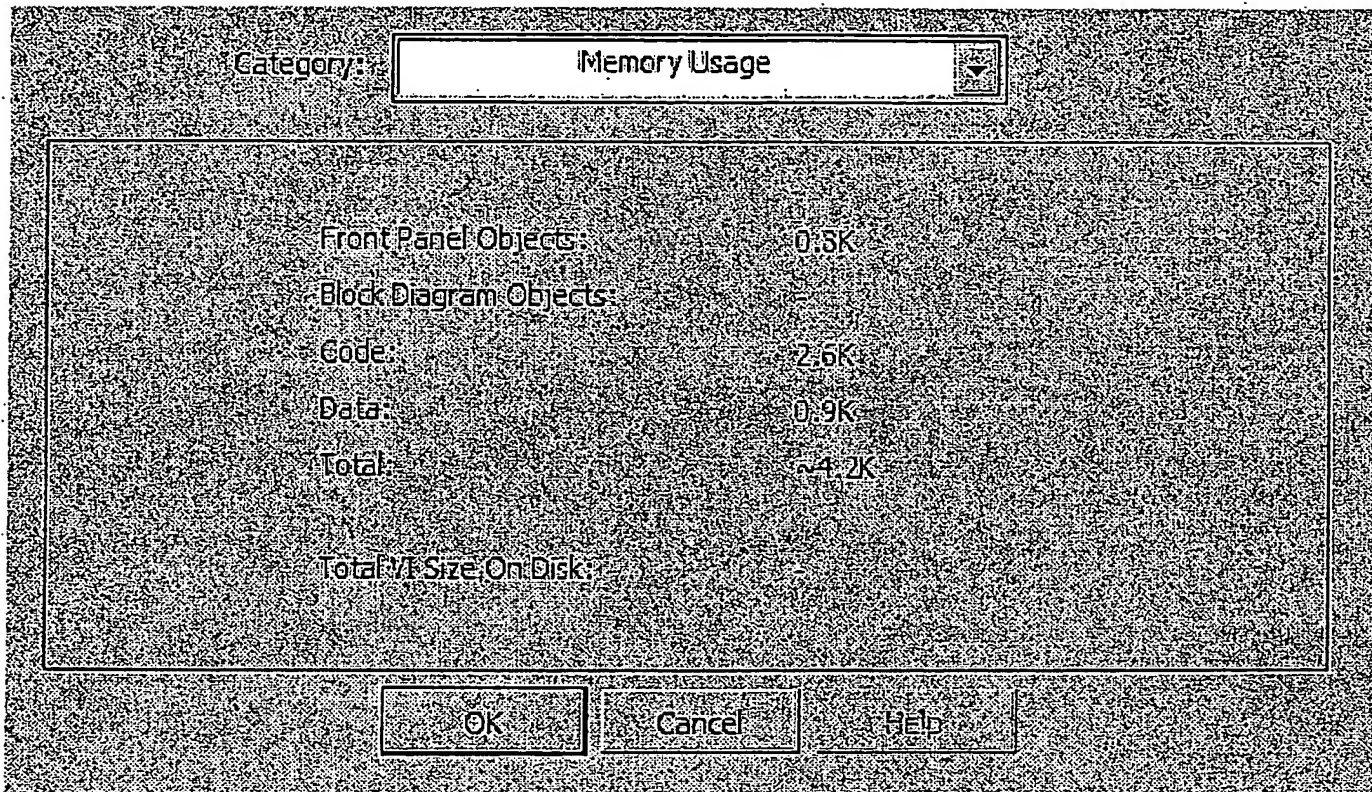


Fig. 21

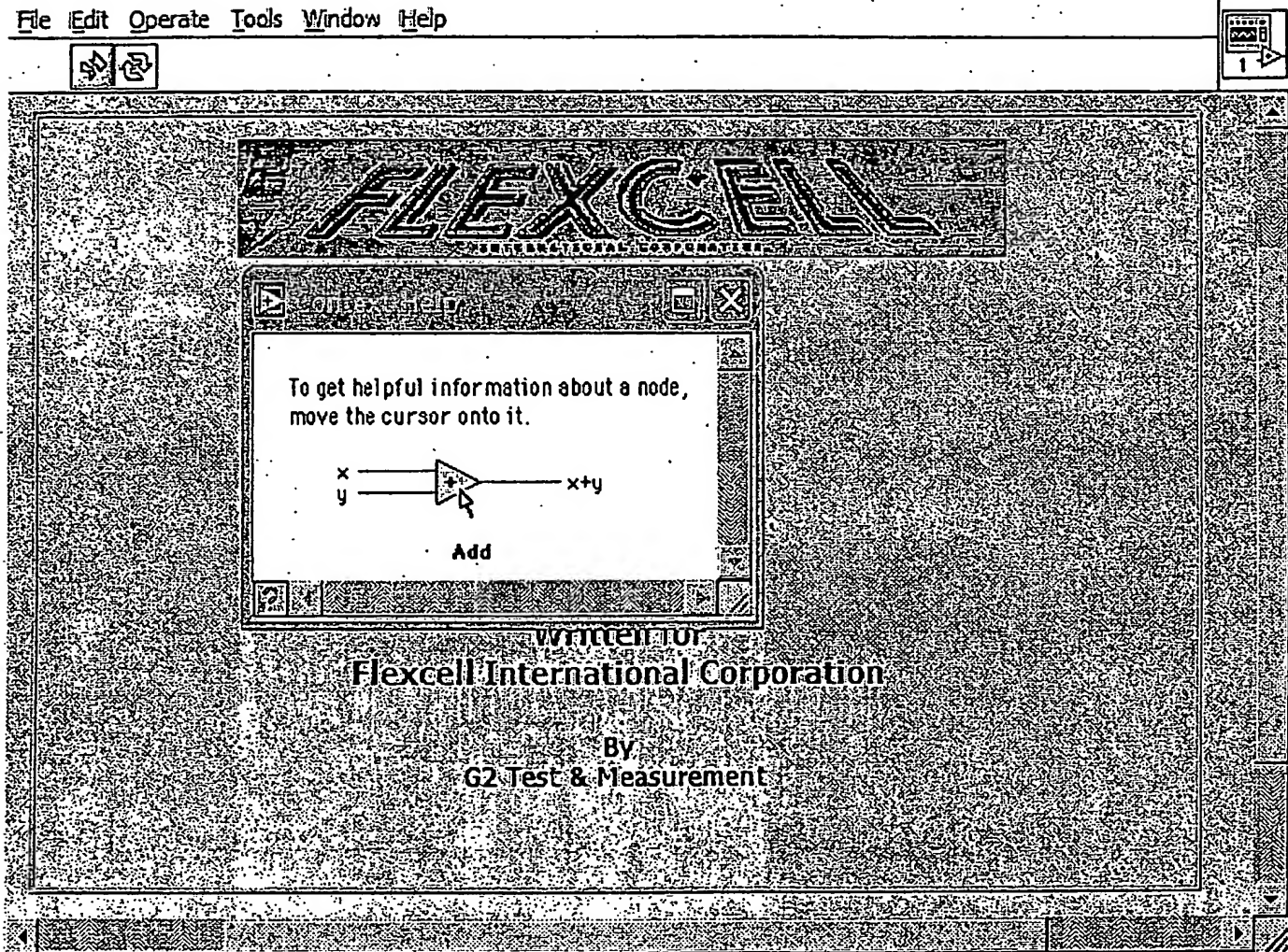


Fig. 22

"Automated System for Imaging Artificial Tissue..."

Albert J. BANES et al.

Attorney Docket No. 717-031951

Patent Information

For the most current list of patents covering this product, please refer to ni.com/legal/patents.

The LabVIEW software is covered by one or more of the following Patents:

United States Patent No(s): 4,901,221; 4,914,568; 5,291,587; 5,301,301;
5,301,336; 5,475,851; 5,481,740; 5,481,741; 5,497,500; 5,504,917;
5,533,988;
5,610,828; 5,652,909; 5,732,277; 5,734,863; 5,737,622; 5,764,546;
5,784,275;
5,821,934; 5,847,953; 5,905,649; 5,920,479; 5,974,254; 5,990,906;
6,064,812;
6,064,816; 6,102,965; 6,138,270;
D384051; D387750; D384050; D384052
European Patent No(s): 0242131
Japanese Patent No(s): 3,016,783
Canadian Patent No(s): 1285655

Various other software products may be included with this version of LabVIEW. If any software products listed below are included, they are covered by various Patents as follows:

The LabVIEW Signal Processing Toolset is covered by one or more of the following Patents:

U.S. Patent No(s): 5,353,233; 6,108,609
European Patent No(s): 0632899
Japanese Patent No(s): 2,697,957

The LabVIEW Datalogging and Supervisory Control Module is covered by one or more of the following Patents:

U.S. Patent No(s): 5,966,532; 6,053,951

LabVIEW Real Time is covered by one or more of the following Patents:

U.S. Patent No(s): 6,173,438

The LabVIEW PID Control Toolset is covered by one or more of the following Patents:

U.S. Patent No(s): 6,081,751

The IVI Driver Toolset is covered by one or more of the following Patents:

U.S. Patent No(s): 5,963,726; 6,035,156

The NI-VISA software is covered by one or more of the following Patents:

U.S. Patent No(s): 5,724,272; 5,710,727; 5,847,955; 5,640,572; 5,771,388;
5,627,983; 5,717,614

The NI-DAQ software is covered by one or more of the following Patents:

U.S. Patent No(s): 5,619,702; 6,067,534; 6,096,094; 6,052,743; 6,143,438;
5,926,775; 5,937,530; 6,073,205

The NI-433 or NI-433.2 (NI-GPIB) software is covered by one or more of the following Patents:

U.S. Patent No(s): 5,974,541; 5,964,892; 5,958,028; 5,987,530; 6,073,205

The NI-FBUS software, including one or more of the NI-FBUS Configurator software or the NI-FBUS Monitor software, is covered by one or more of the following Patents:

U.S. Patent No(s): 5,854,890; 5,796,721; 5,850,523; 5,971,581; 6,141,596;
6,076,952; 5,978,850;

"Automated System for Imaging Artificial Tissue..."

Albert J. BANES et al.

Attorney Docket No. 717-031951

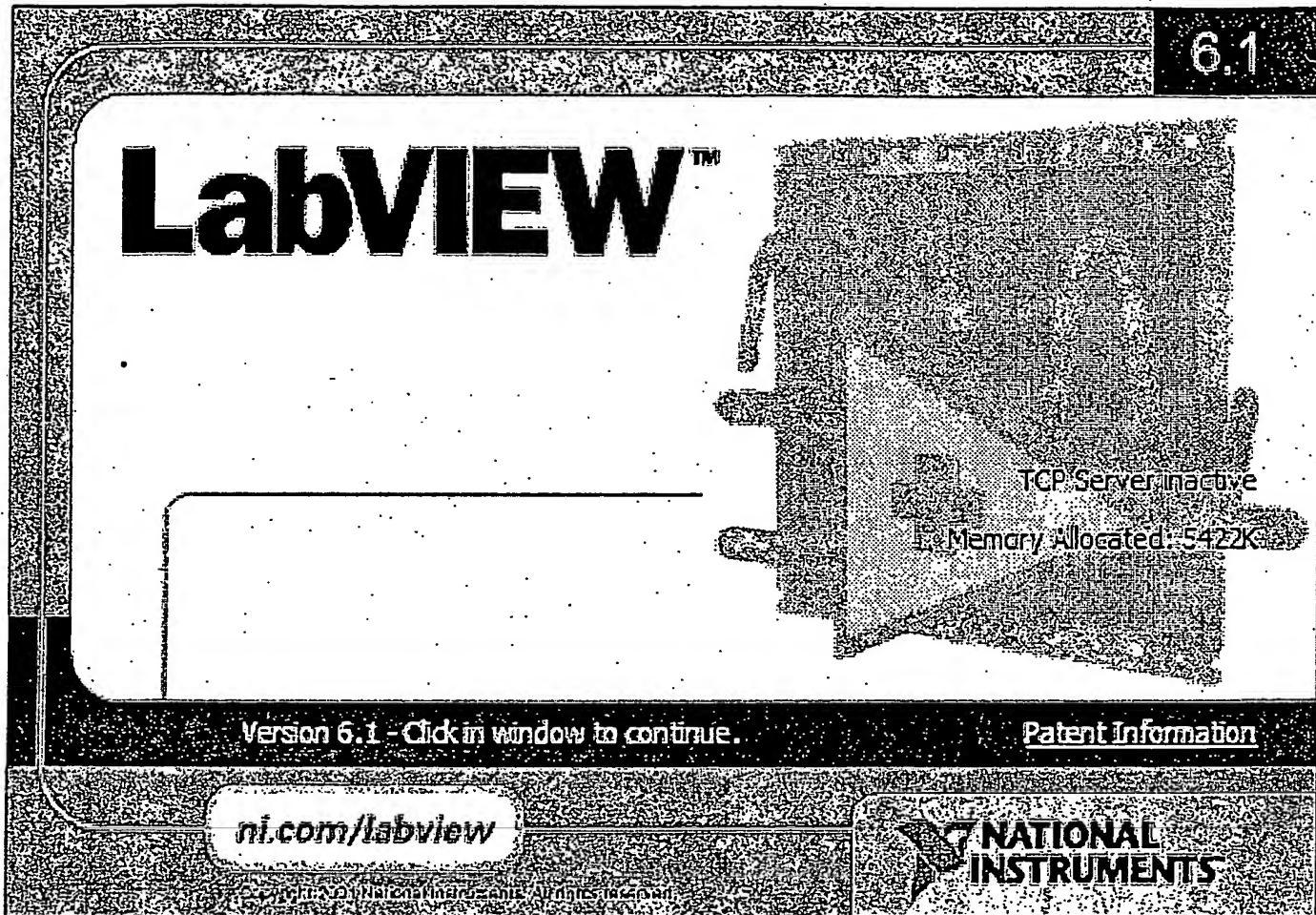


Fig. 21

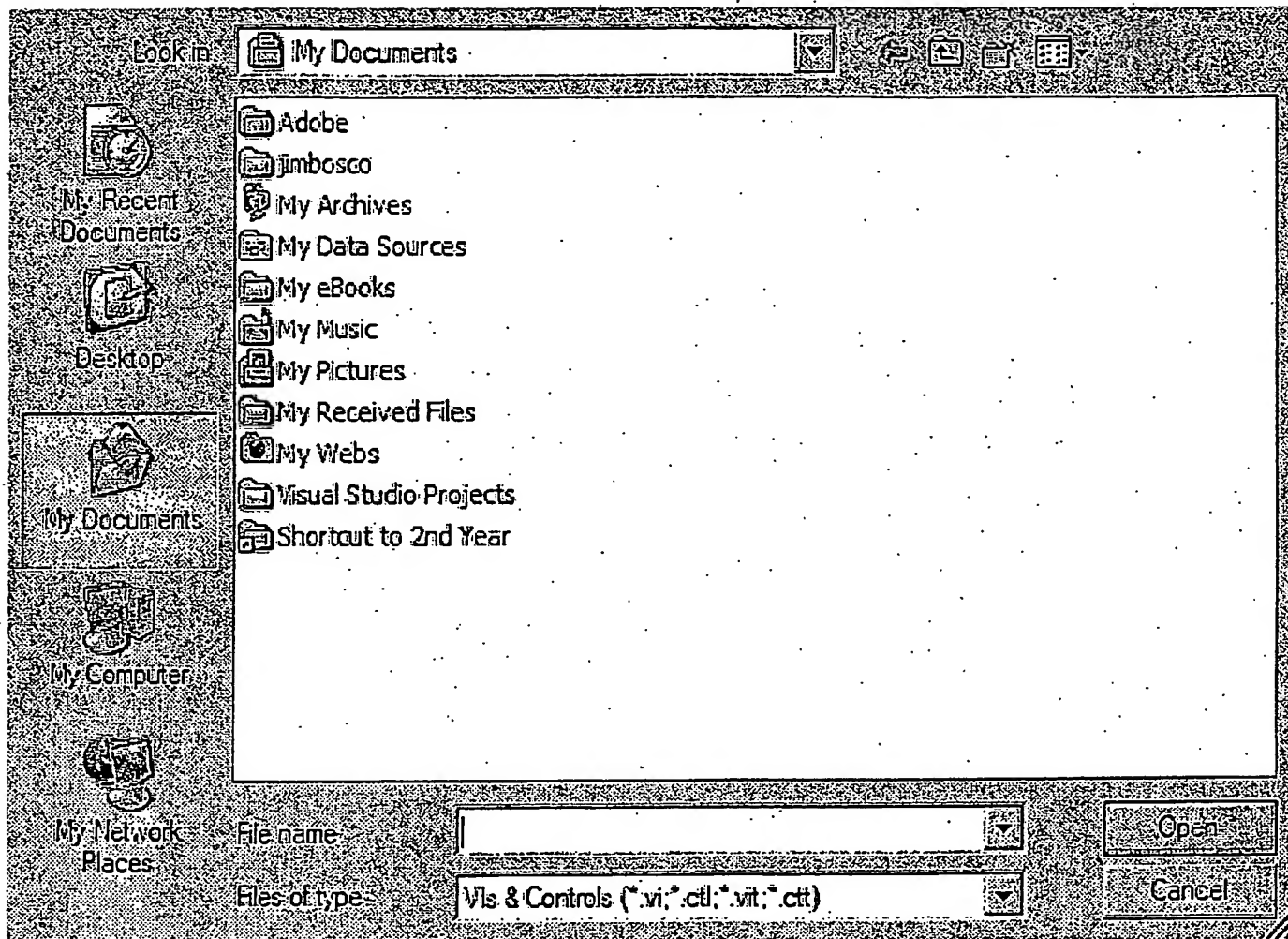


Fig. 25

☒ Print header (name, date, page #)

☐ Use default margins from preferences

☐ Custom margins

Left: 0.00

Right: 0.00

Top: 0.00

Bottom: 0.00

Units: Inches

cm

Help

OK Cancel Printer setup ...

Fig. 26

"Automated System for Imaging Artificial Tissue..."

Albert J. BANES et al.

Attorney Docket No. 717-031951

Acquire.exe is a Windows utility that transfers images from TWAIN devices to files in BMP or JPEG format. Because it is controlled by command-line parameters, Acquire.exe is suitable for use from the DOS box as a spawned sub-program, or from a desktop icon.			About...
Usage: ACQUIRE [options] [filename]			
The options are described in the table below. Case is not important. /INCHES and /inches are equivalent. If no options are given, /H/NOSCAN is assumed. filename is the name or full path of the saved image file. If no extension is given, .bmp or .jpg is added. If no filename is given, the user is prompted for it.			
Option	Definition	Example	
/AUTOBRIGHTn	Set automatic brightness 1=On, 0=Off	/autobright1	
/ADF	Enable automatic feeding of documents	/adf	
/Bn	Set Brightness to n. (very device specific)	/b500	
/BMP	Write .bmp files (default)	/bmp	
/BW	Request 1-bit black & white	/BW	
/cm	Set units to centimeters	/cm	
/Cn	Set Contrast to n. (very device specific)	/C0	
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/FEEDER	Select document feeder	/Feeder	
/GAMMAN	Set gamma to n	/Gamma1.8	
/GRAY	Request 8-bit grayscale	/Gray	
/H or /?	Display this help text (and exit)	/?	
/HIDE	Ask the imaging device to hide its dialog	/hide	
/HIGHn	Set highlight value = n	/high248	
/Hn	Set Height to scan (in units)	/H10	
/I or /Inches	Set units to inches (default)	/i	
/JPEG	Write .jpg files (JPEG/JFIF format) with quality 75 (good)	/jpeg	
/JPEGn	Write .jpg files with quality n (1..100)	/jpeg50	
/LOG	Log internal TWAIN activity to c:\twain.log	/log	
/N or /Nn	Scan multiple images (up to n)	/n /n3	
note: Multiple images are saved to filename1, filename2, ...			
/NOINDIC	Request no progress indicators	/noindic	
/NOSCAN	Don't scan	/noScan	
/NOUI	Request no scan dialog (Same as /HIDE above)	/noUI	
/PAPER=s	Select paper size: A3,A4,A5,B4,B5,B6,LETTER,LEGAL	/paper=84	
/PAPER=n	Select paper type n (see TWSS_* in TWAIN.H)	/Paper=3 [letter]	
/PIXELS	Set units to pixels	/pixels	
/REFL	Set light path to Reflective	/refl	
/RGB	Request 24-bit color	/rgb	
/Rn or /R=n	Set Resolution to n in pixels per unit	/r200	
/SCAN	Scan an image, even if other options indicate not	/scan	
/SHADOWn	Set shadow value = n	/shadow10	
/S=s	Use source whose name contains the string s	/s=Fujitsu	
/SS	Display the TWAIN Select Source dialog (and exit)	/SS	
/Tn	Set Threshold to n	/T128	
/TRANS	Set light path to Transmissive	/trans	
/V	Display version information (and exit)	/v	
/Wn	Set Width to scan (in units)	/W6.2	
/Xn	Set left edge of the scan area (units from left)	/X1.5	
/Yn	Set top edge of the scan area (units from top)	/Y7.25	

Fig. 27

MATERIALS and METHODS

Human tendon internal fibroblasts (HTIF, 2×10^5 cells/100 μ l/ specimen) were plated in linear, tethered, collagen gels in TissueTrain™ culture plates (Figure 1).

After 2 hours, when the gels had solidified, the culture plates were removed from the FX-4000TT Tissue Train™ Culture System and placed on the glass of a Plustek OpticPro U24 flatbed scanner, in the incubator (Figure 2).

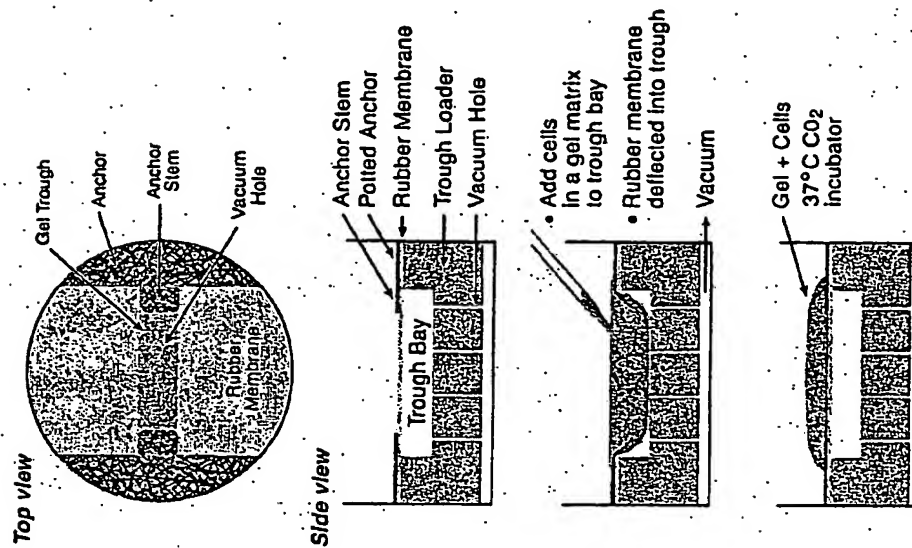


Figure 1. Specimen preparation

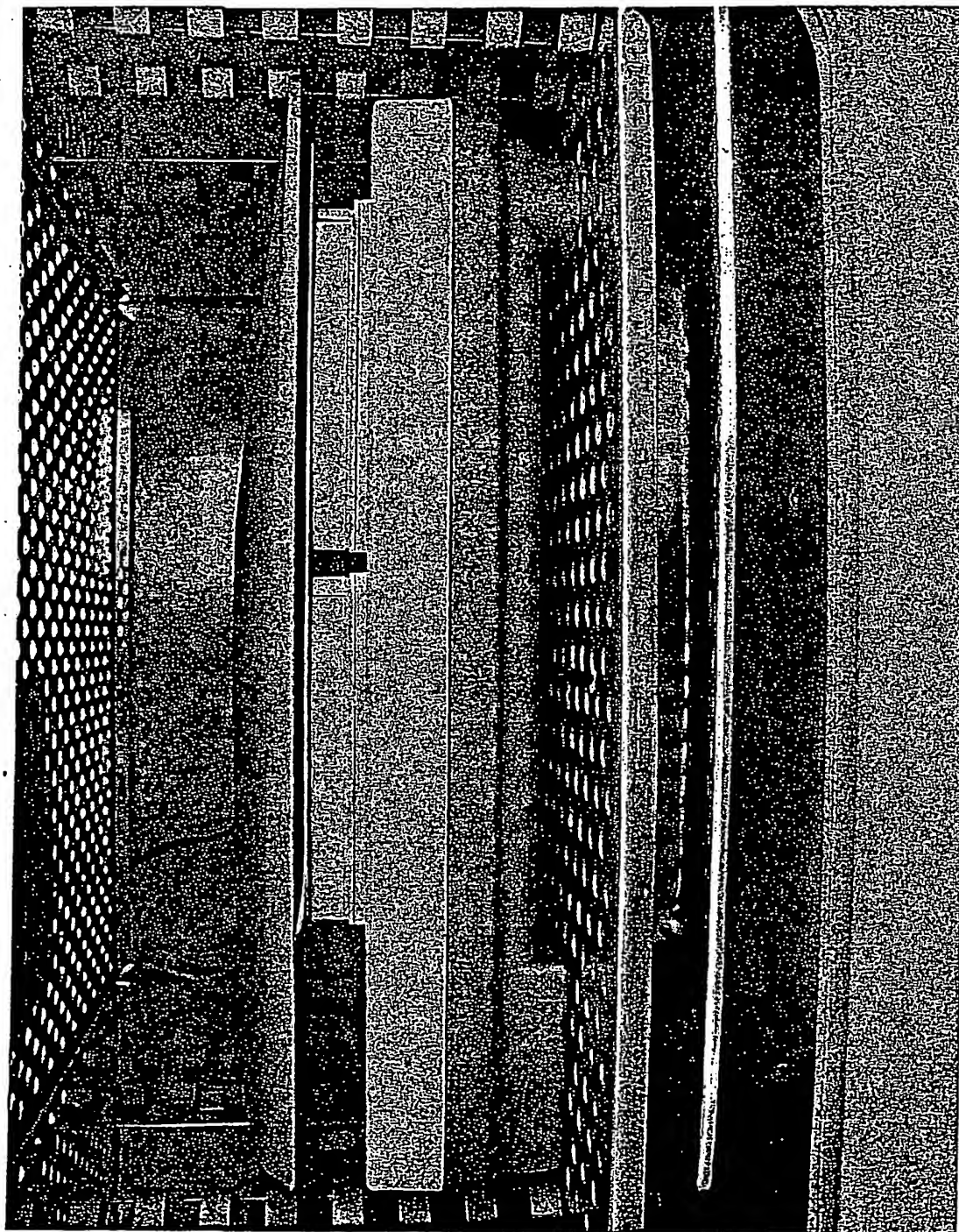


Figure 2: Culture plates prepared for scanning in the incubator.

MATERIALS and METHODS

- Using the custom program, ScanFlex™ (Flexcell International Corp., patent pending), the scanner was configured to collect images every hour for the first 4 hours of each day and every 2 hours for the remainder of the day, for 4 days.
- Culture plates were only removed from the incubator once/day to change the medium.
- Images were imported into SigmaScan software to quantify the area of each gel at each time point.

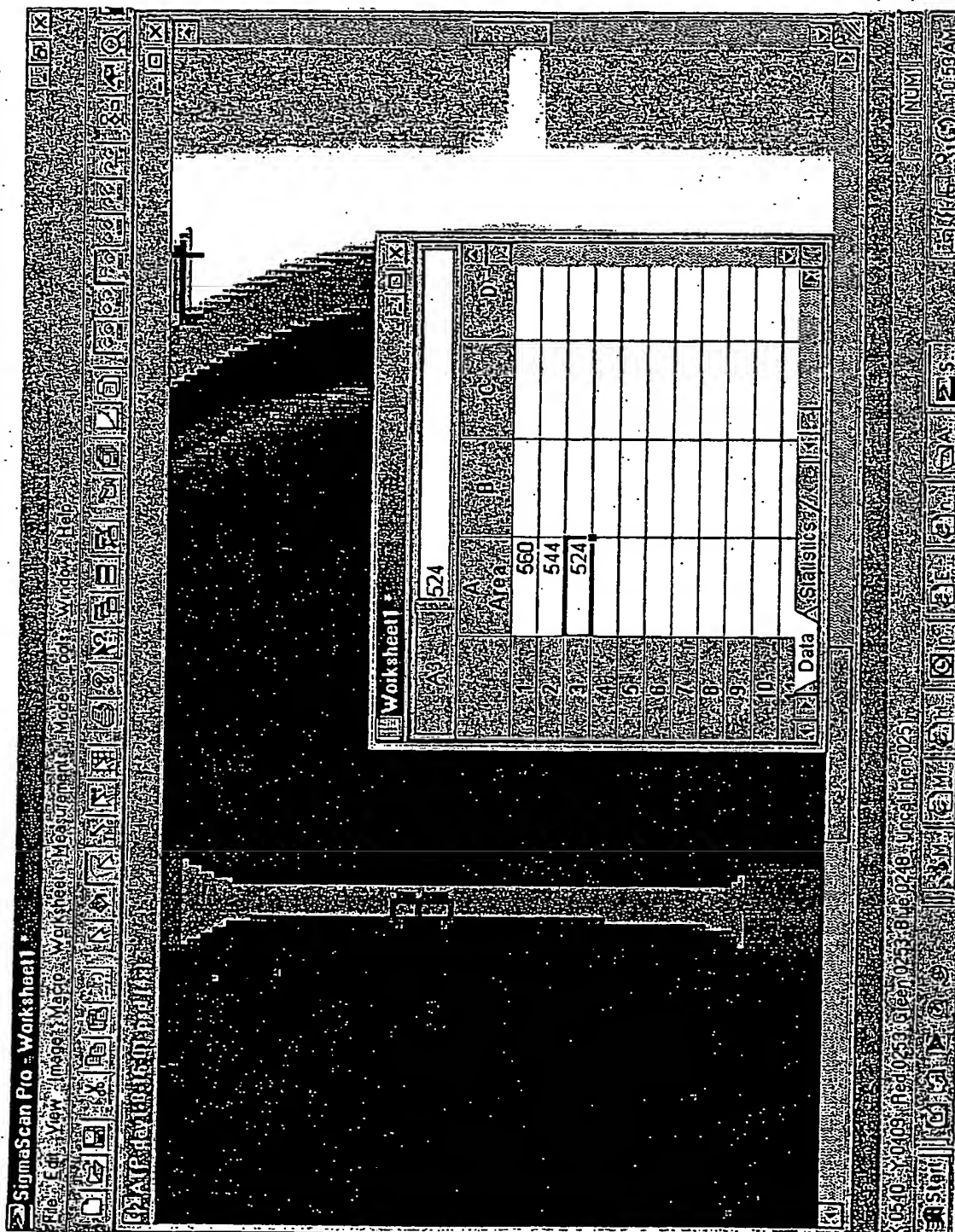


Figure 3. Gel area analysis using Sigma Scan software.

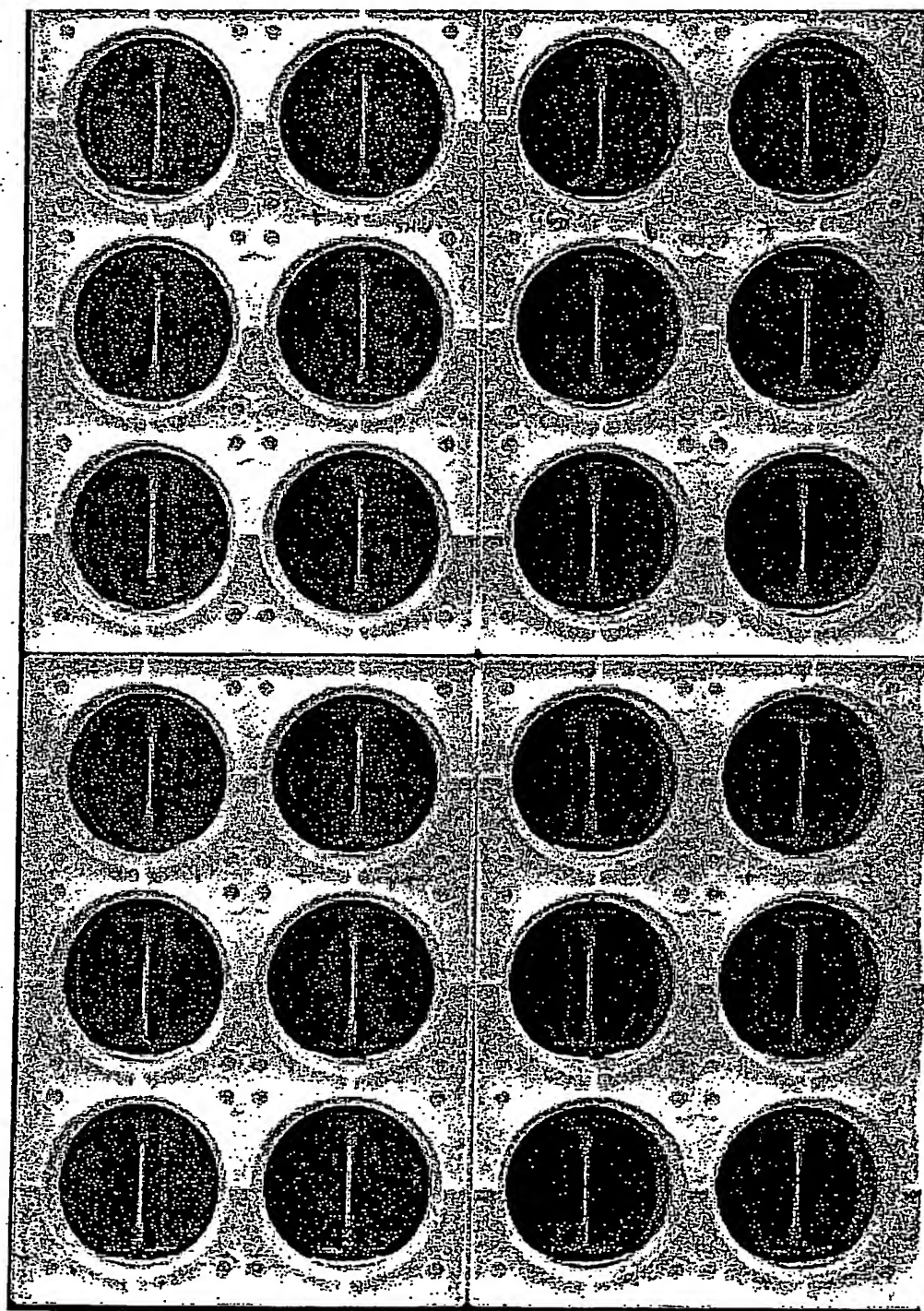


Figure 4. Scan of four Tissue Train™ culture plates taken in the incubator.

RESULTS

Gels experienced the greatest rate of contraction (58.6%) in the first 24 hours, and continued contracting to a total of 73.6% by the end of Day 4.

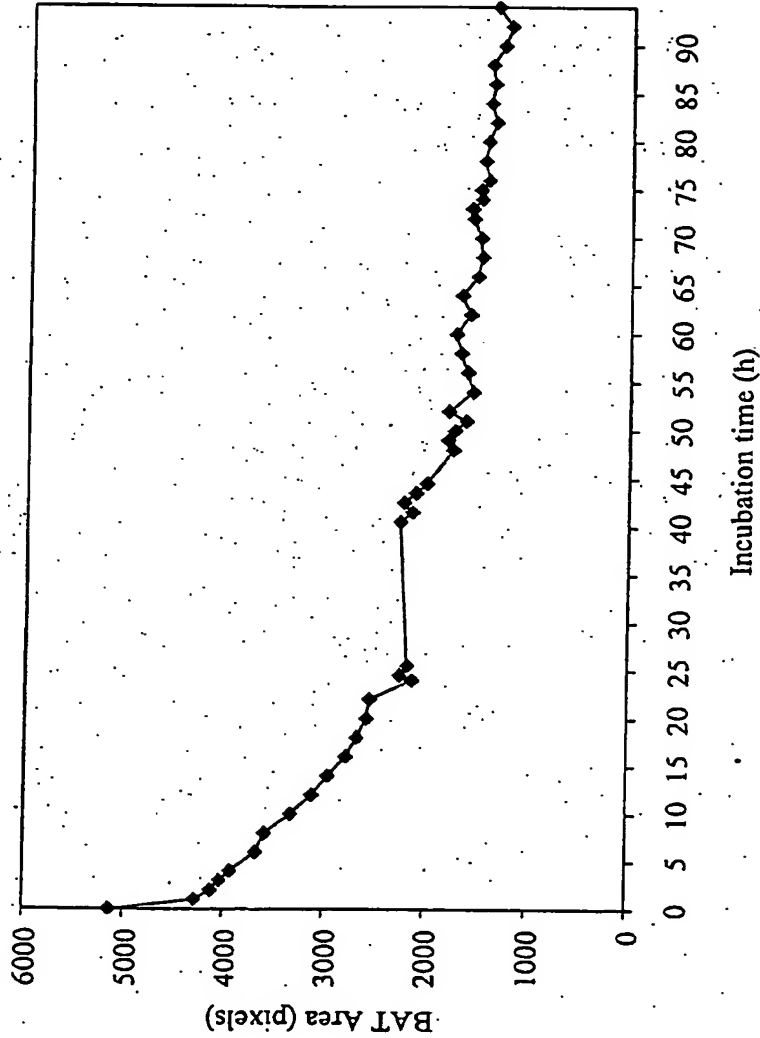


Figure 5. Contraction curve for gels from time of plating through day 4.

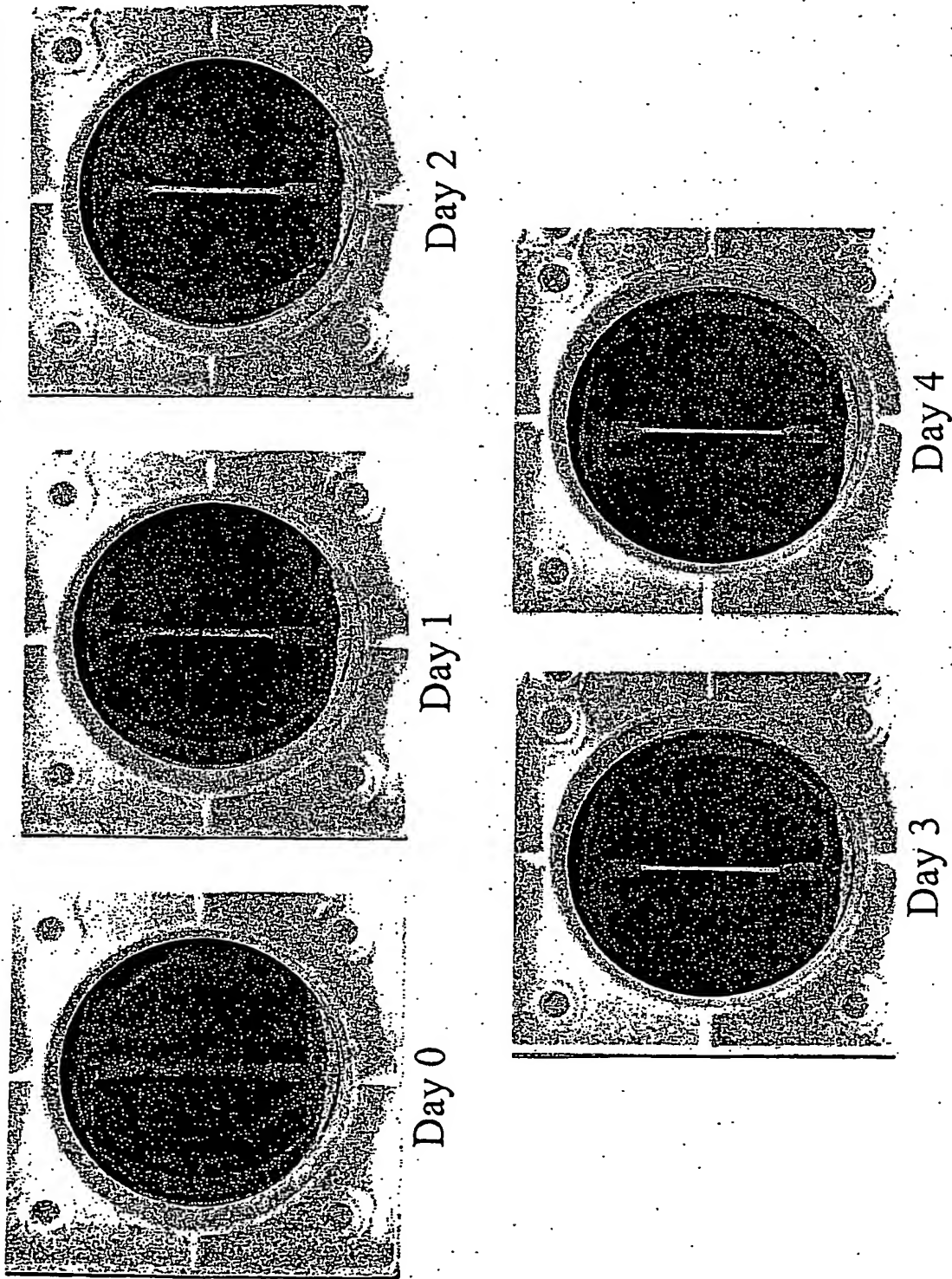


Figure 6: Specimen contraction from time of plating through Day 4.

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